Postnatal selective suppression of lipoprotein lipase gene expression in brown adipose tissue (relative to the expression of the gene for the uncoupling protein) is not due to adrenergic insensitivity: a possible specific inhibitory effect of colostrum

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The levels of mRNA coding for the uncoupling protein (UCP) and for lipoprotein lipase (LPL) were monitored in the brown adipose tissue of newborn rat pups. At 5 h after birth, the mRNA levels of UCP and LPL were high in pups exposed singly to 28 °C and low in pups kept singly at thermoneutrality (36 °C); in pups staying with the dam, the UCP mRNA levels were intermediate. However, the LPL mRNA levels were lower in pups staying with the dam than in pups at 36 °C, implying that factors additional to environmental temperature influenced LPL gene expression. Injection of noradrenaline into pups at thermoneutrality (36 °C) led to increases in UCP and LPL gene expression, but noradrenaline injections had no further effect in cold-exposed pups. The adrenergic effects were mediated via β-adrenergic receptors. The cold-induced increases in both UCP and LPL gene expression were abolished by the β-adrenergic antagonist propranolol. Thus differences in adrenergic responsiveness could not explain the differential expression of the UCP and LPL genes observed in pups staying with the dam. The presence of a physiological suppressor was examined by feeding single pups at 28 °C with different foods: nothing, water, Intralipid, cow's milk, rat milk and rat colostrum. None of these agents led to suppression of UCP gene expression, but colostrum led to a selective suppression of LPL gene expression. It was concluded that the genes for UCP and LPL were responsive to adrenergic stimuli immediately after birth, and it is suggested that a component of rat colostrum can selectively suppress LPL gene expression.

INTRODUCTION

One challenge that a newborn mammal has to face is the adaptation to a new and colder thermal environment. The extra heat needed to protect the newborn against hypothermia is the product of brown-adipose-tissue activity (reviewed in [1]). Heat production in brown adipose tissue results from the combustion of fatty acids. Although many enzymes are involved in this process and may show postnatal increases in expression in the tissue (e.g. the acyl-CoA dehydrogenases [2]), it is generally recognized that it is the amount of the uncoupling protein (UCP; thermogenin) [3,4] that is the rate-limiting factor for non-shivering thermogenesis [5]. In accordance with the postnatally increased demand for heat production, the level of UCP mRNA increases ~ 10-fold during the first 24 h of extrauterine life of the newborn rat [6–9]. This increase is not ontogenically determined, but is the result of a physiologically induced response to a decreased environmental temperature [8–11]. As a consequence of the increase in the amount of mRNA coding for UCP, the amount of UCP in the tissue increases [10–17], as does the thermogenic capacity of the newborn [18] (for review see [1]).

However, in order to ensure a constant supply of fatty acids as a substrate for prolonged heat production, a parallel increase in the capacity for uptake of fatty acids is essential. The fatty acids are taken up from the circulation as a result of the activity of lipoprotein lipase (LPL) [19]. There is a postnatal increase in LPL activity in brown adipose tissue [20,21], and there is also evidence that LPL is functionally active in the newborn. This may be deduced from the shift in the composition of the fatty acids in the tissue triacylglycerols that is observed. In the fetus, the triacylglycerols have a fatty acid composition compatible with them being synthesized within the tissue; after birth, a high fraction of the fatty acids are polyunsaturated, reflecting a transfer from the dam, via the milk, of fatty acids originally synthesized in plants [22–25]. Despite these indications for a postnatal activation of a functional LPL in the tissue, there is practically no increase in the level of mRNA coding for LPL during normal postnatal development [8,9], in contrast with what is the case for UCP.

This raises two questions. One concerns the origin of the LPL activity found postnatally in the brown adipose tissue of the pups; we shall not examine this question in the present investigation. The second is regulatory: in which way is the differential level of expression of the genes for UCP and LPL regulated in the newborn pup? The low expression of the LPL gene during early normal postnatal development (with the dam) is especially confounding, since the brown-adipose-tissue cells possess the ability to increase LPL gene expression if the pups are exposed to a cold environment (without the dam) [8].

Thus, during normal postnatal development, an increased expression of the UCP gene, but not of the LPL gene, is seen; a cold stimulus may, however, induce the expression of both genes. In the present investigation we address the question why LPL and UCP gene expression is not induced in parallel during normal early postnatal life.

MATERIALS AND METHODS

Animals

Pregnant Sprague–Dawley rats were obtained from a local supplier (Alab, Stockholm, Sweden) and housed at 22 °C. The

Abbreviations used: LPL, lipoprotein lipase; UCP, uncoupling protein.
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rats had free access to food [rat pellets (Ewos R3), containing 27% of the energy as protein, 12% as fat, and 60% as carbohydrate] and water and were on a 12 h/12 h light/dark cycle.

Near term, the dams were checked every second hour, and the start and the end of delivery were recorded. Delivery lasted for ~ 1.5 h. In all experiments, the end of delivery was taken as zero time; this necessarily induced a ~ 1.5 h difference between the age of the first and last pup born, and therefore a different degree of exposure to the environmental conditions of the nest before the experiment was initiated. However, this seemed to have only marginal effects on the results. The average weight of the pups was 6 g.

Experiments

Generally, the experiments were performed during the first 5 h after the end of delivery. The pups were exposed to different environmental conditions and different pharmacological treatments, as detailed in the legends to the individual Figures and Tables. The pups were then decapitated, and the interscapular depot of brown adipose tissue was dissected out and immediately frozen on solid CO_2. The samples were stored at −80 °C for not more than 1 week before isolation of RNA (see below).

Injections

Where indicated, the animals were injected subcutaneously (50 μl in the leg fold) with freshly prepared saline solutions of the following agents obtained from Sigma (doses given in μmol of the agent and mg of the parent compound per kg body weight): 3 (if not otherwise indicated) μmol of noradrenaline (1 mg of noradrenaline bitartrate); 3 μmol of isoprenaline (0.96 mg of isoprenaline bitartrate); 4.5 μmol of phenylephrine (0.92 mg of phenylephrine HCl); 100 μmol of DL-propranolol (30 mg of DL-propranolol HCl); 10 μmol (4 mg) of phentolamine (obtained from Ciba–Geigy).

Food

Where indicated, the pups were fed artificially in the following way. The pups were transferred to 28 °C; some of these pups were not fed. The others received water, Intralipid [the commercial 10% Intralipid solution (KabiVitrum), enriched with 48 mM lactose], cow's milk (commercial pasteurized, 3.5% fat) or rat milk (obtained by hand-milking anaesthetized lactating dams on day 4 after delivery; the litter was withdrawn 5 h before milking). The pups at 28 °C were fed twice, at 1 and 3 h, through a plastic pipette tip (40–80 μl of each solution at each time) and were decapitated at 5 h. The presence or absence of milk/liquid in the stomach was checked in each pup. The estimated amount of milk that a 1-day-old pup normally obtains is 0.02 g/h per pup [26]; the amount artificially administered was thus similar to what is normally fed to the pups. Certain pups were given access to colostrum by being allowed to remain with the dam for 2 h after delivery; they were then transferred to 28 °C and were decapitated in parallel with the other pups, 3 h after the transfer. Care was taken that the handling etc. of all pups was as identical as possible, except for the food given.

RNA preparation and determination of mRNA levels

Total RNA was prepared from the frozen tissue as previously described [7,8,27,28], with a guanidine hydrochloride method involving ethanol precipitation. The amount of RNA obtained was determined by spectrophotometry at 260 nm; the A_{260}/A_{280} ratios were always higher than 1.8. The purity of the preparation was generally checked on agarose minigels. The total amount of RNA obtained from the brown adipose tissue of one pup ranged from 30 to 120 μg.

The amount of UCP mRNA, LPL mRNA or actin mRNA was estimated by analysing slot-blots of 4 or 10 μg of total RNA, as previously described [7,8,28], with nick-translated cDNA probes for UCP [27], LPL [29] or β-actin. After hybridization of the slot-blots and overnight exposure of the film, the intensity of the autoradiograms was evaluated in a laser densitometer (LKB 2202 Ultroscan or Molecular Dynamics 300A Computing Densitometer). RNA isolated from the brown adipose tissue of a cold-exposed (24 h) adult rat and from 24 h-old pups were used as reference standards, for both UCP and LPL. All related samples were always hybridized and densitometrically analysed in parallel, and statistical analysis was performed on the densitometric values. For the representation in Figures and Tables, the arbitrary densitometric values were normalized based on the mean expression levels of the appropriate controls.

RESULTS AND DISCUSSION

Effect of environmental conditions on the expression of the genes for UCP and for LPL

In Table 1, the effects of different postnatal environmental conditions on the levels of mRNA coding for UCP and for LPL are shown. The normal postnatal development (allowing the pups to remain with the dam at 22 °C) may be compared with the effect of transfer of the pups to a defined ambient temperature.

In the pups that remained with the dam under standard animal-house conditions for the first 5 h after birth, there was only a tendency to a small increase in the level of UCP mRNA. If the pups were transferred to the relative cold of 28 °C, a significant increase in UCP mRNA levels was observed. If the pups were transferred to a thermoneutral temperature (36 °C) [30], the UCP mRNA level tended to decrease. The implication

Table 1 Effect of environmental conditions on the postnatal changes in the expression of the UCP and LPL genes

Immediately after the end of delivery (zero time), newborn rat pups from 6 dams (13.5 ± 1.1 pups per litter) were either immediately decapitated (0 h value) or were exposed for 5 h to one of three environmental conditions: they remained with the dam under standard animal-house conditions (22 °C), or they were transferred to single cages at an ambient temperature of either 36 or 28 °C. Care was taken to maintain high humidity in the 36 °C incubator. After these 5 h, the pups were decapitated, total RNA was isolated, and the levels of UCP and LPL mRNA were determined in all samples, as described in the Materials and methods section. All related samples were blotted, hybridized and densitometrically analysed in parallel. Logarithmic transformations of the densitometric data (including the individually calculated LPL/UCP ratios) were submitted to one-way analysis of variance (ANOVA) after testing for homogeneity of variance with Barlett's procedure. After the ANOVA [which for all three parameters showed significant effects of treatment ($P < 0.001$)], significant differences between mean values were identified with the protected least significant difference (LSD) test. In the Table, the densitometric values were normalized by being expressed relative to the mean value observed at time 0 h (100%). Points are means ± S.E.M. of 6–8 pups: *** indicates significant difference between the indicated level and the level at zero time ($P < 0.001$).

	UCP	LPL	LPL/UCP
0 h	100 ± 17	100 ± 6	1.00 ± 0.12
5 h			
With dam	121 ± 7	104 ± 6	0.75 ± 0.09
At 28 °C	203 ± 27***	475 ± 47***	1.88 ± 0.11***
At 36 °C	53 ± 9	165 ± 7***	2.38 ± 0.33***

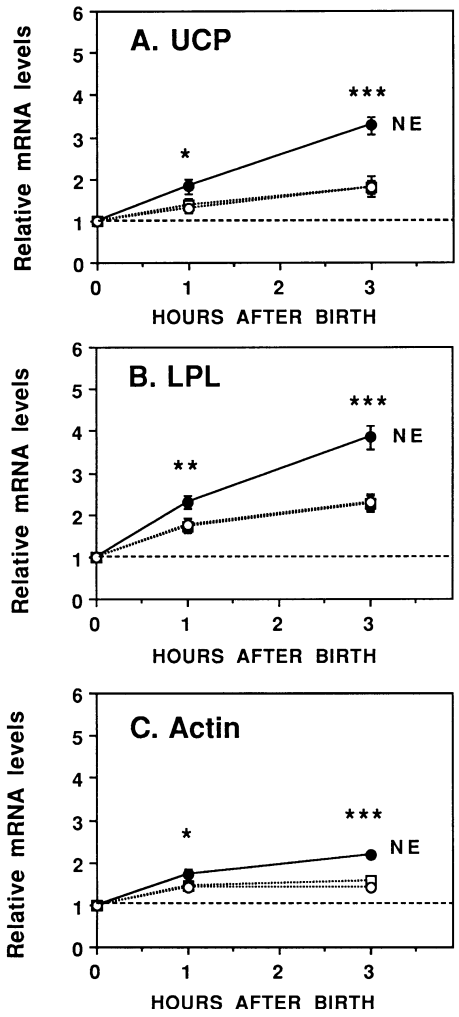

Figure 1 Effect of noradrenaline injection on the postnatal changes in the expression of the UCP and LPL genes in newborns remaining with their dams

Eight dams were used (litter size 10.4 ± 0.7). For each litter, the pups were withdrawn from the dam at zero time; one pup was taken at this time, and the rest were injected subcutaneously, either with 2.3 μmol of noradrenaline per kg body weight (NE, ●) or with saline (○), or not injected at all (□). The pups were placed again with the dam; the dam accepted the injected pups, making no difference compared with the others, all being fed and nursed. After 1 or 3 h, the pups were removed and analysed as described in the Materials and methods section. Results are means ± S.E.M. from 6–8 pups (if not visible, the S.E.M. was smaller than the size of the symbol): *, ** and *** indicate significant differences between the level in the noradrenaline-injected and the saline-injected pups ($P < 0.05$, < 0.01 and < 0.001, respectively; Student's t test).

is that the pups nested with the dam at 22 °C experienced less cold than when they were exposed singly to 28 °C (but slightly more so than singly at 36 °C), and that they had a potential ability to respond to the thermal environment to a greater extent than is normally necessary.

The LPL mRNA level remained unchanged when the pups remained with the dam. It increased dramatically when the pups were exposed to 28 °C, which was in accordance with a simple thermal control of LPL gene expression and in agreement with what we had observed previously [8]. However, the LPL mRNA levels were higher in the pups which were left singly at thermoneutral temperature (36 °C) than in those which remained with the dam at a lower temperature. For this observation, there can be no explanation based exclusively on temperature effects.

The difference in the regulation of the expression of the LPL and UCP genes is even more evident when the LPL/UCP ratio is calculated (Table 1). In the presence of the dam, this ratio tends to decrease, but in the absence of the dam, the LPL mRNA level increases even more than the UCP mRNA level.

Thus, from these experiments, it was clear that immediately after birth the pups were able to respond to alterations in environmental conditions. The expression of the UCP and the LPL genes was, however, not similarly controlled.

Adrenergic induction of gene expression in newborn rat pups

In adult mice and rats, the increase in UCP gene expression observed in the cold is due to sympathetic adrenergic stimulation of the brown fat cells, mainly via β-adrenergic pathways [3,4,31]. Also the cold-induced LPL gene expression in the brown adipose tissue of adult rats is β-adrenergically regulated, although this gene has the ability to respond to both insulin and noradrenaline [32]. Brown adipose tissue possesses β-adrenergic receptors already on the first postnatal day, and these receptors are coupled to stimulation of adenylate cyclase [33]. However, it has been suggested that the increase in UCP gene expression *in utero* may be induced via non-adrenergic mechanisms [9,34], and that the ability to respond to physiological stimuli with increased UCP gene expression may not be present before birth, but may develop thereafter [9]. There is at present no published evidence that adrenergic stimulation can induce either UCP or LPL gene expression in the brown adipose tissue of the newborn pup. We therefore investigated the ability of the newborn pups to respond to adrenergic stimulation in this way. These experiments were performed with pups staying with the dams, i.e. under conditions where the expression is fairly stable (cf. Table 1).

As injections in themselves may be perceived as stressful by the pups, and as this could induce an endogenous sympathetic stimulation of the tissue, we used two types of controls: non-injected pups and saline-injected pups. However, the values obtained for the saline and non-injected groups of pups were superimposable (Figure 1), indicating that the stress caused by the injection itself did not appreciably influence the expression.

In the non-injected pups and in the pups injected with saline, UCP mRNA levels increased to some extent during the first 3 h after birth [principally in agreement with the results shown above (Table 1)]. Injection of noradrenaline led to a significant increase in the level of UCP mRNA (Figure 1A).

In the untreated pups, LPL gene expression also increased after birth, but noradrenaline injection led to a markedly higher level of expression (Figure 1B).

As a control, we also examined the effect of adrenergic stimulation on β-actin gene expression. A small increase was observed 1 h after birth (Figure 1C), and injection of noradrenaline also caused a small increase. That pharmacological or physiological conditions may marginally affect the levels of β-actin mRNA in brown adipose tissue has previously been observed [35,36]. However, such variations, including those observed here, were always small as compared with the changes observed in UCP and LPL mRNA levels. It is nevertheless likely that the small variations represent true changes in β-actin gene expression and do not reflect different relative yields of mRNA or any other artefact.

Based on the observations of the effects of noradrenaline injection (Figure 1), it was clear that the brown adipose tissue of the newborn rat pup already possessed the ability to transduce an adrenergic signal into increased expression of both the UCP and

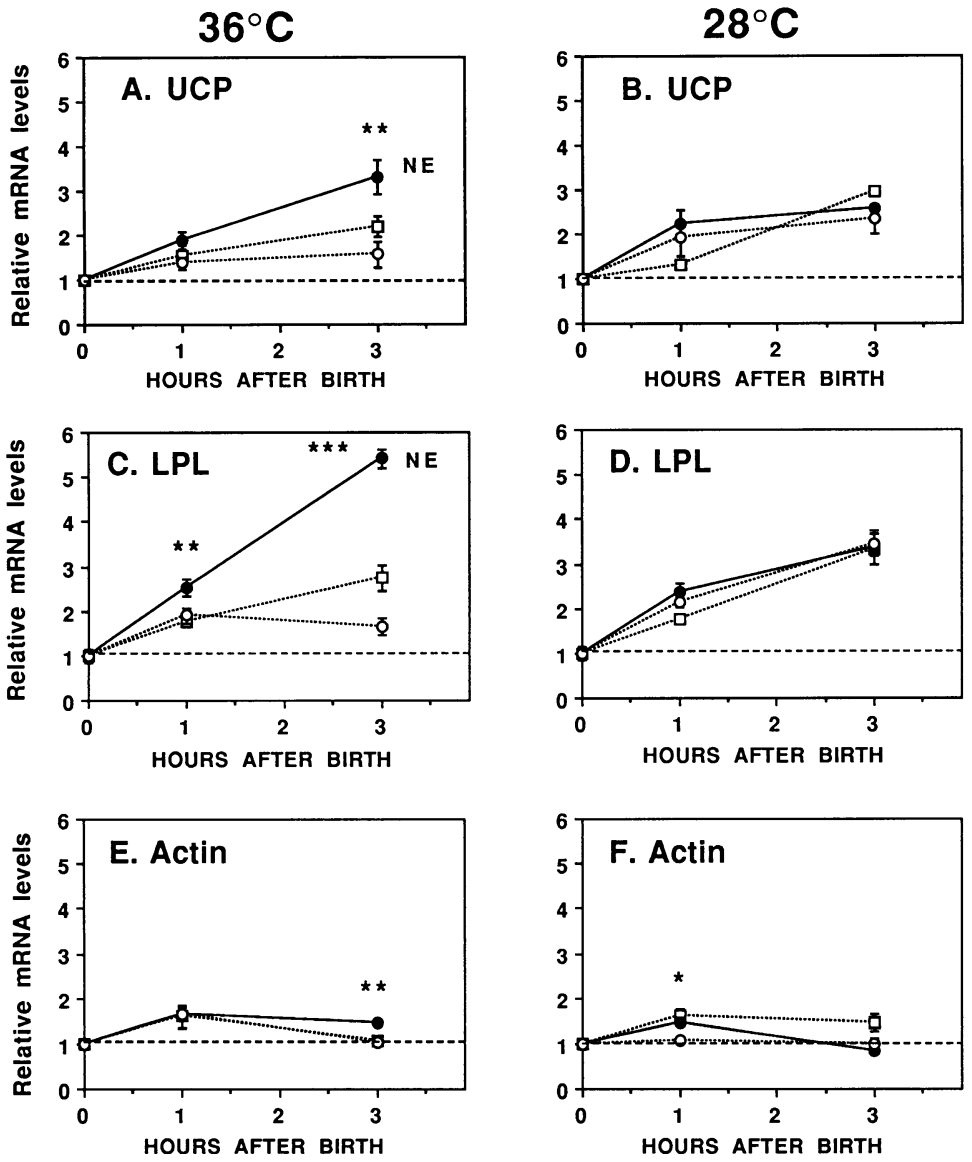

Figure 2 Effect of noradrenaline injection on the postnatal changes in the expression of the UCP and LPL genes in newborns exposed to different environmental temperatures

Eight dams were used (12.2 ± 0.7 pups per litter). The litters were withdrawn from the dam at zero time, and half of each litter was transferred to single cages at 36 °C and the other half to single cages at 28 °C. From both groups, two pups were injected with 2.4 μmol of noradrenaline per kg body weight (NE, ●), two pups were injected with saline (○) and the last two were not injected (□). The pups were taken individually at zero time (non-injected only) and 1 and 3 h later. Values are means ± S.E.M. from 4–8 pups in each group (where not shown, the S.E.M. was smaller than the size of the symbol): *, ** and *** indicate significant effects of noradrenaline versus saline injection, with the same *P* values as in Figure 1.

the LPL gene. A differential sensitivity to adrenergic stimulation could therefore not explain the differential intensity of expression observed during normal postnatal development (Table 1).

Adrenergic stimulation mimics the effect of cold exposure on UCP and LPL gene expression

Although the pups were able to respond to noradrenaline, the adrenergic pathway may not be the pathway utilized physiologically for the induction of enhanced gene expression in the cold. If the adrenergic pathway is used, it would be expected that in thermoneutral pups (at 36 °C) noradrenaline should mimic the effect of exposure to cold (28 °C), but in cold-exposed pups, the effect of noradrenaline should be much decreased or absent (as the adrenergic pathway would already be stimulated physiologically).

In pups transferred to thermoneutrality (36 °C), UCP mRNA levels did not change much during the first 3 h (Figure 2A). The injection of noradrenaline led to a clear increase in UCP mRNA levels. That this response mimicked the effect of cold exposure may be seen by comparing the level obtained after noradrenaline stimulation with that obtained in pups exposed to cold (28 °C) (Figure 2B). There was no further noradrenaline effect in the pups in the cold (Figure 2B).

LPL mRNA levels increased somewhat in the thermoneutral pups after birth (Figure 2C). Noradrenaline injection led to a large increase in the level of LPL mRNA; the level even exceeded that in the pups in the cold (Figure 2D), where a continuous

Table 2 Effect of adrenergic agonists on the expression of the genes for UCP and LPL in pups at thermoneutrality

Six dams were used (11.8 ± 0.6 pups per litter). The litters were withdrawn from the dam at zero time, and four pups of each litter were injected with the indicated agents and immediately placed at thermoneutral temperature (36 °C). The pups were taken individually 4 h later; brown adipose tissue was dissected out, and mRNA levels were estimated as described in the Materials and methods section. The mean levels of mRNA coding for UCP or for LPL in pups exposed to 36 °C without treatment were set at 100% and the other values expressed relative to this. Results are thus means ± S.E.M. of 6 determinations (pups) in each group: *, ** and *** indicate levels of gene expression significantly different from that in the non-treated pups ($P < 0.05$, < 0.01 and < 0.001 respectively; Student's unpaired t test).

	UCP	LPL
Basal (36 °C)	100 ± 15	100 ± 17
+ Noradrenaline	343 ± 32***	289 ± 32***
+ Isoprenaline	226 ± 23**	226 ± 38**
+ Phenylephrine	166 ± 38	154 ± 20
+ Isoprenaline + phenylephrine	172 ± 2*	252 ± 43**

Table 3 Effect of adrenergic antagonists on the cold-induced increase in UCP and LPL gene expression

Six dams were used (11.8 ± 0.6 pups per litter). The litters were withdrawn from the dam at zero time. Some pups were transferred to single cages at thermoneutral temperature (36 °C) or at 28 °C; others were injected with the indicated adrenergic antagonists before being transferred to single cages at 28 °C. Pups were decapitated 4 h later, and mRNA levels analysed as described in the Materials and methods section. The mean level of UCP or LPL mRNA observed in the untreated pups at 28 °C was set at 100% and the other values are expressed relative to this. Values are means ± S.E.M. from 3–6 pups in each group: *, ** and *** indicate significant differences between untreated pups at 28 °C and the indicated group, with the same P values as in Figure 1.

	UCP	LPL
36 °C	54 ± 8**	66 ± 8*
28 °C	100 ± 8	100 ± 11
28 °C + propranolol	65 ± 10*	50 ± 3**
28 °C + phentolamine	64 ± 18	82 ± 27
28 °C + propranolol + phentolamine	23 ± 4***	43 ± 11*

increase in LPL mRNA levels was found. In the pups in the cold, no further effect of noradrenaline injection was observed.

The effects of cold exposure and of noradrenaline on β-actin mRNA levels were small (Figures 2E and 2F).

From the experiments described above, it was clear that adrenergic stimulation of pups at thermoneutrality was able to mimic the effect of cold exposure, and that cold exposure masked the ability of injected noradrenaline to promote (further) gene expression. These observations were compatible with an adrenergic mediation of the stimulation of UCP and LPL gene expression in cold-exposed pups.

Pharmacological characterization of the noradrenaline effect

To identify the adrenergic-receptor type involved in the stimulation of the expression of the UCP and LPL genes, the effect of noradrenaline (which stimulates both α- and β-adrenergic receptors) was compared with the effect of selective β-adrenergic stimulation (with isoprenaline) or α-adrenergic stimulation (with phenylephrine). The effects were tested in pups transferred to thermoneutrality (36 °C), so that the endogenous expression of UCP and LPL should be minimal (cf. Table 1). The injection of either noradrenaline or isoprenaline visibly affected the brown adipose tissue in the pups, observed as an intensified blood flow similar to that observed during exposure to cold. In this respect, phenylephrine had a much lower effect, if any.

In agreement with the results above, the UCP mRNA level was increased 4 h after the injection of noradrenaline (Table 2). Isoprenaline also had an effect, although the magnitude was lower than that of noradrenaline ($P < 0.01$). Phenylephrine in itself had no significant effect, and there was no statistically significant effect of phenylephrine on the effect of isoprenaline. These results, implying mainly a β-adrenergic pathway for the stimulation of UCP gene expression in the pups, were not identical with those obtained in adult mice [28], where a synergistic interaction between α- and β-adrenergic stimulation was found.

As also expected from the above results (Figure 1), the injection of noradrenaline led to an increase in the level of LPL mRNA (Table 2). Isoprenaline had an effect that was not statistically different from that of noradrenaline. Phenylephrine led to a small increase that was not statistically significant ($P < 0.01$), and it did not add to the effect of isoprenaline. These results, implying mainly a β-adrenergic pathway for the stimulation of gene expression in the pups, were similar to those observed in adult rats [32].

Inhibition of the cold-induced increase in UCP and LPL gene expression by adrenergic antagonists

Whereas the results of the adrenergic-agonist experiments (Figures 1 and 2 and Table 2) were compatible with a β-adrenergic pathway mediating the cold-induced gene expression in the newborn pups, they do not demonstrate that this pathway is the one actually utilized. Non-adrenergic pathways have been implied in other circumstances [37], and such pathways could be responsible for the increase in gene expression in the cold; adrenergic stimulation may then not be able to increase gene expression further. We therefore investigated whether the cold-induced increase in gene expression could be blocked by adrenergic antagonists. As administration of α-adrenergic blockers to intact animals may be detrimental, due to vasodilation and a large decrease in blood pressure, the dose of α-antagonist has to be kept relatively low, and, as there is reason to assume that the β-receptors involved in the adrenergic response (Table 2) are of the β_3-type, which are characterized by a relatively low sensitivity to propranolol [38], the dose of propranolol used has to be kept relatively high.

The increase in UCP mRNA levels induced by cold exposure at the time point examined represented a doubling of the level in the pups at thermoneutrality (Table 3). Injection of propranolol before cold exposure decreased the UCP mRNA level to a level which was not different from that observed in the pups at thermoneutrality. The decrease obtained with the α-antagonist phentolamine was more varied and was not statistically significant, although the mean decrease was similar to that observed with propranolol. The simultaneous injection of propranolol and phentolamine led to a suppression of UCP gene expression below the level observed in the pups at thermoneutrality. However, this double antagonist treatment was detrimental to the pups. The results indicate that it is likely that the increase in UCP gene expression induced by cold exposure is mediated predominantly through a β-adrenergic pathway, even in the newborn rat pups.

The results concerning LPL gene expression were even clearer (Table 3). Propranolol fully abolished the effect of cold exposure on the level of gene expression, whereas phentolamine was without effect. The combined injection of propranolol and phentolamine did not potentiate the effect of propanolol. Thus,

Table 4 Effect of feeding on the increase in UCP and LPL gene expression observed at 28 °C

Six dams were used (the same dams as in Table 1). For each dam the litter was taken from the dam at zero time and distributed as follows: five pups were housed singly at 28 °C, and one at 36 °C. The rest were placed either back with the dam or with a non-lactating dam found appropriate to nurse pups. One of the pups replaced to the dam was transferred to 28 °C 2 h later (after being fed with colostrum by the dam) and thus remained at 28 °C for 3 h (+Colostrum). The pups placed with the non-lactating dam either remained fasting for 5 h, or were fed with Intralipid solution. The pups at 28 °C were fed with water, Intralipid solution, cow's milk or rat milk as described in the Materials and methods section. After 5 h, the pups were decapitated and mRNA levels determined as described in the Materials and methods section. All related samples were blotted, hybridized and densitometrically analysed in parallel. The densitometric data (including the individually calculated LPL/UCP ratios) were submitted to one-way analysis of variance (ANOVA) after testing for homogeneity of variance with Barlett's procedure. Logarithmic transformation of the data generally yielded homogeneity in cases where this criterion was not met by the raw data. After the ANOVA [which for all three parameters showed very significant effects of treatment ($P < 0.001$)], significant differences between mean values were identified with the protected least-significant-difference (LSD) test. In the Table, the values are normalized by setting the mean levels of mRNA coding for UCP or for LPL (and the ratio LPL/UCP) in pups exposed to 28 °C without liquid at 100% (or 1.00) and expressing the other values relative to this. Results are means ± S.E.M. of 4–6 determinations (pups) in each group: *, ** and *** indicate levels of gene expression or ratios significantly different from those observed in pups at 28 °C without liquid ($P < 0.05$, < 0.01 and < 0.001 respectively).

	UCP	LPL	LPL/UCP
Single, cold (28 °C)			
+Nothing	100 ± 13,	100 ± 13	1.00 ± 0.06
+Water	110 ± 13	110 ± 10	1.17 ± 0.15
+Intralipid	91 ± 5	99 ± 4	1.01 ± 0.05
+Cows milk	108 ± 14	97 ± 5	1.14 ± 0.14
+Rat milk	165 ± 8***	146 ± 12**	0.98 ± 0.10
+Colostrum	140 ± 6**	72 ± 9*	0.57 ± 0.09**
Single, thermoneutral (36 °C)			
+Nothing	26 ± 4***	34 ± 2***	1.26 ± 0.18
With dams (22 °C)			
Own dam	60 ± 4**	22 ± 1***	0.40 ± 0.05***
Non-lactating dam	37 ± 4***	43 ± 1***	0.92 ± 0.28
Non-lactating dam, + Intralipid	45 ± 3***	39 ± 6***	0.74 ± 0.04

the cold-induced increase in LPL gene expression was mediated through a β-adrenergic pathway, even in the newborn rat pups.

Does colostrum contain an inhibitory factor for LPL gene expression?

From the experiments above, it was clear that LPL gene expression (as well as UCP gene expression) in the newborn pup may be increased due to cold exposure and that this increase occurs via an adrenergic pathway. As UCP gene expression is increased during normal development [8,9], probably via the adrenergic pathway, it would seem unavoidable that LPL gene expression should be induced in parallel. Thus, the question remained why a low LPL gene expression was observed under conditions when UCP gene expression was stimulated, i.e. during normal postnatal development. One possiblity would be the existence of a physiological suppressor.

One effect of the presence of the dam is the availability of food. We therefore investigated whether any component of the food could suppress UCP and LPL gene expression. For this, we supplied food artificially to cold-exposed pups (Table 4).

Concerning UCP, liquid or food in any form (water, Intralipid solution, cow's milk, rat milk or rat colostrum) was unable to suppress cold-induced gene expression. The only foods that had a significant effect were rat milk and colostrum, which both led to a higher level of UCP mRNA being present. Principally, this positive effect is in accordance with the idea that homologous milk is best for the development of the pups [39].

We also confirmed that it was not the milk, but rather the nesting with the dam, which led to lower expression of the UCP gene during normal postnatal development. For this, we transferred some pups to dams who were no longer lactating. Despite the fact that these pups did not obtain food from the dams, UCP gene expression was decreased to approximately the same level as in pups at thermoneutrality (Table 4). It was therefore concluded that the postnatal increase in the expression of the UCP gene was not secondary to food deprivation, but was most likely an effect of the ambient temperature experienced by the pup (in agreement with the implication of our previous results [8]).

In the case of LPL gene expression, there was no significant suppression by water, Intralipid solution or cow's milk, and rat milk had a positive effect, just as it had for UCP gene expression. However, an interesting effect of rat colostrum was noted. LPL gene expression was suppressed in rats which had received rat colostrum, whereas the degree of UCP gene expression was higher. As shown in the last column of Table 4, this is the only occasion in which the effect of different liquids on UCP and LPL gene expression significantly diverged. This implies that access to colostrum may selectively suppress LPL gene expression. It may be noted that this effect must be ascribed specifically to the colostrum, as it was not found in milk from rats which had been lactating for 4 days.

We also investigated whether nesting with the dam led to selective suppression of LPL gene expression. We found that in the pups transferred to non-lactating dams, the differential degree of expression of the genes for UCP and for LPL was not observed (Table 4).

It may therefore be suggested that the postnatal suppression of the expression of the gene for LPL may be due to a component of rat colostrum. The identification of this component is a future challenge.

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REFERENCES

1 Nedergaard, J., Connolly, E. and Cannon, B. (1986) in Brown Adipose Tissue (Trayhurn, P. and Nicholls, D. G., eds.), pp. 152–213, Edward Arnold, London
2 Hainline, B. E., Kahlenbeck, D. J., Grant, J. and Strauss, A. W. (1993) Biochim. Biophys. Acta **1216**, 460–468
3 Klaus, S., Casteilla, L., Bouillaud, F. and Ricquier, D. (1991) Int. J. Biochem. **23**, 791–801
4 Nedergaard, J. and Cannon, B. (1992) New Compr. Biochem. **23**, 385–420
5 Cannon, B., Nedergaard, J. and Sundin, U. (1981) in Survival in the Cold: Hibernation and other Adaptations (Musacchia, X. J. and Jansky, L., eds.), pp 99–120, Elsevier/North-Holland, Amsterdam
6 Ricquier, D., Bouillaud, F., Toumelin, P., Mory, G., Bazin, R., Arch, J. and Penicaud, L. (1986) J. Biol. Chem. **261**, 13905–13910
7 Obregon, M. J., Pitamber, R., Jacobsson, A., Nedergaard, J. and Cannon, B. (1987) Biochem. Biophys. Res. Commun. **148**, 9–14
8 Obregon, M. J., Jacobsson, A., Kirchgessner, T., Schotz, M. C., Cannon, B. and Nedergaard, J. (1989) Biochem. J. **259**, 341–346
9 Giralt, M., Martin, I., Iglesias, R., Vinas, O., Villarroya, F. and Mampel, T. (1990) Eur. J. Biochem. **193**, 297–302
10 Luis, A. and Cuezva, J. M. (1989) Biochem. Biophys. Res. Commun. **159**, 216–222
11 Mouroux, I., Bertin, R. and Portet, R. (1990) J. Dev. Physiol. **14**, 337–342
12 Sundin, U. and Cannon, B. (1980) Comp. Biochem. Physiol. **65B**, 463–471
13 Bazin, R., Eteve, D. and Lavau, M. (1984) Biochem. J. **221**, 241–245
14 Felipe, A., Villarroya, F. and Mampel, T. (1988) Biol. Neonate **53**, 105–112
15 Porras, A., Penas, M., Fernández, M. and Benito, M. (1990) Eur. J. Biochem. **187**, 671–675

16 Körtner, G., Schildhauer, K., Petrova, O. and Schmidt, I. (1993) Am. J. Physiol. **264**, R1017–R1023
17 Zimmerberg, B., Brown, A. P., Lee, H. H. and Slocum, R. D. (1993) Alcohol (N.Y.) **10**, 149–153
18 Moore, R. E. and Underwood, M. C. (1963) J. Physiol. (London) **168**, 290–317
19 Borensztajn, J. (1987) Lipoprotein Lipase, Evener, Chicago
20 Cryer, A. and Jones, H. M. (1978) Biochem. J. **174**, 447–452
21 Galan, X., Llobera, M. and Ramirez, I. (1993) Biol. Neonat. **64**, 295–303
22 Cogneville, A. M., Cividino, N. and Tordet-Caridroit, C. (1975) J. Nutr. **105**, 982–988
23 Ricquier, D. and Hemon, P. (1976) Biol. Neonate **28**, 225–240
24 Senault, C., Solier, M., Beauvallet, M. and Portet, R. (1982) Experientia **38**, 585–587
25 Ohno, T., Ogawa, K. and Kuroshima, A. (1992) Int. J. Biometeorol. **36**, 30–35
26 Chalk, P. A. and Bailey, E. (1979) J. Dev. Physiol. **1**, 61–79
27 Jacobsson, A., Stadler, U., Glotzer, M. A. and Kozak, L. P. (1985) J. Biol. Chem. **260**, 16250–16254
28 Jacobsson, A., Nedergaard, J. and Cannon, B. (1986) Biosci. Rep. **6**, 621–631
29 Kirchgessner, T. G., Svenson, K. L., Lusis, A. J. and Schotz, M. C. (1987) J. Biol. Chem. **262**, 8463–8466
30 Thompson, G. E. and Moore, R. E. (1968) Can. J. Physiol. Pharmacol. **46**, 865–871
31 Cannon, B., Bengtsson, T., Dicker, A., Jacobsson, A., Kuusela, P., Thonberg, H., Tvrdik, P., Zhao, J. and Nedergaard, J. (1994) in Thermal Balance in Health and Disease: Recent Basic Research and Clinical Progress (Zeisberger, E., Schönbaum, E. and Lomax, P., eds.), pp. 87–102, Birkhäuser, Basel
32 Mitchell, J. R. D., Jacobsson, A., Kirchgessner, T. G., Schotz, M. C., Cannon, B. and Nedergaard, J. (1992) Am. J. Physiol. **263**, E500–E506
33 Svoboda, P., Skobisova, E. and Drahota, Z. (1984) Physiol. Bohemoslov. **33**, 97–103
34 Cannon, B., Connolly, E., Obregon, M. J. and Nedergaard, J. (1988) in The Endocrine Control of the Fetus (Kunzel, W. and Jensen, A., eds.), pp. 306–320, Springer-Verlag, Berlin
35 Herron, D., Néchad, M., Rehnmark, S., Nelson, B. D., Nedergaard, J. and Cannon, B. (1989) Am. J. Physiol. **257**, C920–C925
36 Jacobsson, A., Mühleisen, M., Cannon, B. and Nedergaard, J. (1994) Am. J. Physiol. **266**, R999–R1007
37 Desautels, M. and Dulos, R. A. (1993) Am. J. Physiol. **265**, R103–R110
38 Arch, J. R. S. and Kaumann, A. J. (1993) Med. Res. Rev. **13**, 663–729
39 Casey, C. E. (1989) Proc. Nutr. Soc. **48**, 271–278

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